

CLAIMS

What is claimed is:

1. A method for the determination of the functional effect of a test agent on a nuclear receptor protein or an active fragment thereof comprising the steps of: (A) combining in a first *in vitro* reaction cocktail said nuclear receptor protein or said active fragment thereof fused to a purification facilitating compound; a nuclear
5 receptor coregulator protein or an active fragment thereof fused to an enzyme or a fragment thereof whose activity is simply quantified; a ligand for said nuclear receptor protein; a purification facilitating partner affixed to a solid support; and said test agent; (B) incubating the components of step (A) to allow said components to form a complex; (C) removing said solid support from the remainder of said first *in vitro*
10 reaction cocktail; (D) determining an amount of said complex that was formed by assaying said removed solid support for a first activity of said enzyme or fragment thereof; and (E) assessing whether said test agent functioned as an effector of complex formation by comparing said first activity to a second activity from said enzyme or fragment thereof, recovered from a second *in vitro* reaction cocktail
15 comprising all of the components of step (A) except said test agent, where said second *in vitro* reaction cocktail was subjected to steps (B) to (D).

2. The method as defined in claim 1 wherein said test agent is a protein, peptide, nucleic acid, hormone, cytokine, lipid, carbohydrate, vitamin, mineral, large organic molecule, small organic molecule, non-organic agent or any combination thereof.

3. The method as defined in claim 1 wherein said nuclear receptor is a steroid receptor.

4. The method as defined in claim 1 wherein said nuclear receptor is a non-steroid receptor.

5. The method as defined in claim 1 wherein said nuclear receptor is a peroxisome proliferator-activated receptor, thyroid receptor, estrogen receptor, glucocorticoid receptor, progesterone receptor, androgen receptor, mineralcorticoid

receptor, retinoic acid receptor, retinoid X receptor, vitamin D receptor, orphan
 5 receptor, any fragment thereof or any combination thereof.

6. The method as defined in claim 1 wherein said active fragment of
 said nuclear receptor comprises the ligand binding domain.

7. The method as defined in claim 5 wherein said active fragment of
 said nuclear receptor comprises the ligand binding domain.

8. The method as defined in claim 1 wherein said active fragment of
 said nuclear receptor coregulator comprises one or more LXXLL motifs.

9. The method as defined in claim 1 wherein said purification
 facilitating compound is glutathione-S-transferase, maltose K, influenza
 hemagglutinin, avidin, biotin, FLAG, myc tag, histidine multimers, or any combination
 thereof.

10. The method as defined in claim 1 wherein said purification facilitating
 partner is glutathione, maltose, anti-influenza hemagglutinin antibodies, avidin, biotin,
 anti-FLAG antibodies, anti-myc antibodies, ionic nickel, or any combination thereof.

11. The method as defined in claim 1 wherein said nuclear receptor
 coregulator is a nuclear receptor coactivator.

12. The method as defined in claim 11 wherein said nuclear receptor
 coactivator is a steroid receptor coactivator-1, steroid receptor coactivator-2, steroid
 receptor coactivator-3, transcription intermediary factor 2, glucocorticoid receptor
 interacting protein 1, retinoic acid receptor interacting protein 3, coactivator-
 5 associated arginine methyltransferase 1, peroxisome proliferator-activated receptor
 gamma coactivator-1, peroxisome proliferator-activated receptor gamma
 coactivator-2, p300/CREB binding protein, p300, CREB-binding protein-interacting
 protein, nuclear-receptor co-activator protein, p300/CBP-associated factor,
 alteration/deficiency in activation 3 protein, small nuclear RING finger protein,
 10 thyroid hormone receptor-associated protein 220, NR-binding SET-domain-
 containing protein, any fragment thereof, or any combination thereof.

13. The method as defined in claim 1 wherein said nuclear receptor
 coregulator is a nuclear receptor corepressor.

14. The method as defined in claim 13 wherein said nuclear receptor
 corepressor is nuclear receptor corepressor (N-Cor), small ubiquitous nuclear
 corepressor, silencing mediator for retinoic acid and thyroid hormone receptors,

transcription intermediary factor 2, thyroid hormone receptor uncoupling protein,
 5 calreticulin, repressor of estrogen receptor activity, NR-binding SET-domain-
 containing protein, any fragment thereof, or any combination thereof.

15. The method as defined in claim 1 wherein said solid support is a
 glass bead, cellulose bead, polystyrene bead, sephadex bead, sepharose bead,
 polyacrylamide bead, agarose bead, magnetic bead, multi-well plate, glass reaction
 vessel, or plastic reaction vessel.

16. The method as defined in claim 1 wherein said enzyme is luciferase,
 β -galactosidase, alkaline phosphatase, peroxidase, chloramphenicol acetyl
 transferase or green fluorescent protein.

17. The method as defined in claim 1 wherein said second *in vitro*
 reaction cocktail comprises a control agent known to have an effect on said nuclear
 receptor.

18. The method as defined in claim 5 wherein said second *in vitro*
 reaction cocktail comprises a control agent known to have an effect on said nuclear
 receptor.

19. The method as defined in claim 1 wherein said first *in vitro* reaction
 cocktail comprises multiple nuclear receptors or active fragments thereof
 essentially simultaneously and said method comprises an additional step (f)
 comprising deconvoluting the active nuclear receptor after assessing whether said
 5 test agent functioned as an effector of the nuclear receptor protein.

20. The method as defined in claim 1 wherein said test agent functioned
 as an agonist of complex formation, an antagonist of complex formation, or a ligand
 for said nuclear receptor protein.

21. A high-throughput assay comprising the method as defined in claim
 1 and utilizing multiple *in vitro* reaction cocktails for the determination of the
 functional effect of multiple test agents on a nuclear receptor protein or a fragment
 thereof.

22. A method for the identification of a nuclear receptor ligand,
 comprising the steps of: (A) combining in a first *in vitro* reaction cocktail a nuclear
 receptor protein or an active fragment thereof fused to a purification facilitating
 compound; a nuclear receptor coregulator protein or an active fragment thereof
 5 fused to an enzyme or a fragment thereof whose activity is simply quantified; a

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purification facilitating partner affixed to a solid support; and a test agent; (B) incubating the components of step (A) to allow the components to form a complex; (C) removing said solid support from the remainder of said first *in vitro* reaction cocktail; (D) determining an amount of complex that was formed by assaying said removed solid support for a first activity of the enzyme or fragment thereof; and (E) assessing whether said test agent functioned as a ligand for the nuclear receptor or active fragment thereof by comparing said first activity to a second activity from said enzyme or fragment thereof recovered from a second *in vitro* reaction cocktail comprising all of the components of step (A) except said test agent, where said second *in vitro* reaction cocktail was subjected to steps (B) to (D).

23. The method as defined in claim **22** wherein said test agent is a protein, peptide, nucleic acid, hormone, cytokine, lipid, carbohydrate, vitamin, mineral, large organic molecule, small organic molecule, non-organic agent or any combination thereof.

24. The method as defined in claim **22** wherein said nuclear receptor is a steroid receptor.

25. The method as defined in claim **22** wherein said nuclear receptor is a non-steroid receptor.

26. The method as defined in claim **22** wherein said nuclear receptor is peroxisome proliferator-activated receptor, thyroid receptor, estrogen receptor, glucocorticoid receptor, progesterone receptor, androgen receptor, mineralcorticoid receptor, retinoic acid receptor, retinoid X receptor, vitamin D receptor, orphan receptor, any fragment thereof, or any combination thereof.

27. The method as defined in claim **22** wherein said active fragment of said nuclear receptor comprises the ligand binding domain.

28. The method as defined in claim **26** wherein said active fragment of said nuclear receptor comprises the ligand binding domain.

29. The method as defined in claim **22** wherein said active fragment of said nuclear receptor coregulator comprises one or more LXXLL motifs.

30. The method as defined in claim **22** wherein said purification facilitating compound is glutathione-S-transferase, maltose K, influenza hemagglutinin, avidin, biotin, FLAG, myc tag or histidine multimers.

31. The method as defined in claim **22** wherein said purification facilitating partner is glutathione, maltose, anti-influenza hemagglutinin antibodies, avidin, biotin, anti-FLAG antibodies, anti-myc antibodies, ionic nickel, or any combination thereof.

32. The method as defined in claim **22** wherein said nuclear receptor coregulator is a nuclear receptor coactivator.

33. The method as defined in claim **32** wherein said nuclear receptor coactivator is steroid receptor coactivator-1, steroid receptor coactivator-2, steroid receptor coactivator-3, transcription intermediary factor 2, glucocorticoid receptor interacting protein 1, retinoic acid receptor interacting protein 3, coactivator-associated arginine methyltransferase 1, peroxisome proliferator-activated receptor gamma coactivator-1, peroxisome proliferator-activated receptor gamma coactivator-2, p300/CREB binding protein, p300, CREB-binding protein-interacting protein, nuclear-receptor co-activator protein, p300/CBP-associated factor, alteration/deficiency in activation 3 protein, small nuclear RING finger protein, thyroid hormone receptor-associated protein 220, NR-binding SET-domain-containing protein, any fragment thereof, or any combination thereof.

34. The method as defined in claim **22** wherein said nuclear receptor coregulator is a nuclear receptor corepressor.

35. The method as defined in claim **34** wherein said nuclear receptor corepressor is nuclear receptor corepressor (N-Cor), small ubiquitous nuclear corepressor, silencing mediator for retinoic acid and thyroid hormone receptors, transcription intermediary factor 2, thyroid hormone receptor uncoupling protein, calreticulin, repressor of estrogen receptor activity, NR-binding SET-domain-containing protein, any fragment thereof or any combination thereof.

36. The method of claim **22** wherein said solid support is a glass bead, cellulose bead, polystyrene bead, sephadex bead, sepharose bead, polyacrylamide bead, agarose bead, magnetic bead, multi-well plate, glass reaction vessel or plastic reaction vessels.

37. The method of claim **22** wherein said enzyme is luciferase, β -galactosidase, alkaline phosphatase, peroxidase, chloramphenicol acetyl transferase, and green fluorescent protein.

38. The method as defined in claim **22** wherein said second *in vitro* reaction cocktail comprises a control agent known to be a ligand for said nuclear receptor or active fragment thereof.

39. A high-throughput assay comprising the method as defined in claim **22** and utilizing multiple *in vitro* reaction cocktails for the identification of a ligand for a nuclear receptor protein or a fragment thereof.

40. A method for the determination of a functional effect of a test agent on a peroxisome proliferator-activated receptor protein or a fragment thereof, comprising the steps of: (A) combining in a first *in vitro* reaction cocktail said peroxisome proliferator-activated receptor protein or said fragment thereof fused to the glutathione-S-transferase protein or a fragment thereof; a steroid receptor coactivator-1 protein or a fragment thereof fused to the luciferase protein or a fragment thereof; a GW2331 ligand; glutathione-sepharose beads; and said test agent; (B) incubating the components of step (A) to allow said components to form a complex; (C) removing said glutathione-sepharose beads from the remainder of said first *in vitro* reaction cocktail; (D) determining an amount of said complex that was formed by assaying said removed glutathione-sepharose beads for a first luciferase activity; and (E) assessing whether said test agent functioned as an effector of complex formation by comparing said first luciferase activity to a second luciferase activity recovered from a second *in vitro* reaction cocktail comprising all of the components of step (A) except said test agent, where said second *in vitro* reaction cocktail was subjected to steps (B) to (D).

41. A method for the identification of a ligand for a peroxisome proliferator-activated receptor protein or a fragment thereof, comprising the steps of: (A) combining in a first *in vitro* reaction cocktail said peroxisome proliferator-activated receptor protein or said fragment thereof fused to the glutathione-S-transferase protein or a fragment thereof; a steroid receptor coactivator-1 protein or a fragment thereof fused to the luciferase protein or a fragment thereof; glutathione-sepharose beads; and a test agent; (B) incubating the components of step (A) to allow said components to form a complex; (C) removing said glutathione-sepharose beads from the remainder of said first *in vitro* reaction cocktail; (D) determining an amount of said complex that was formed by assaying

said removed glutathione-sepharose beads for a first luciferase activity; and (E) assessing whether said test agent functioned as a ligand for peroxisome proliferator-activated receptor protein by comparing said first luciferase activity to a second luciferase activity recovered from a second *in vitro* reaction cocktail comprising all of the components of step (A) except said test agent, where said second in vitro cocktail was subjected to steps (B) to (D).

42. A pharmaceutical composition comprising an effector as defined in claim 1 and a pharmaceutically acceptable carrier, vehicle, or diluent.

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